INTERNATIONAL SEARCH REPORT

Information on patent family members

01/08/00

International application No.
PCT/DK 00/00300

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9414963 A1	07/07/94	AU 5699994 A	19/07/94
		BR 9307678 A	31/08/99
		CA 2150837 A	07/07/94
		CN 1090328 A	03/08/94
		CZ 9501578 A	13/12/95
		EP 0679188 A	02/11/95
		HU 71325 A	28/11/95
		HU 9501771 D	00/00/00
		JP 8504588 T	21/05/96
		PL 309388 A	02/10/95
		SK 79595 A	08/11/95
		ZA 9309415 A	15/06/95
WO 9535381 A1	28/12/95	AU 2884595 A	15/01/96
WO 9205249 A1	02/04/92	AT 169671 T	15/08/98
		AU 657278 B	09/03/95
		AU 8617291 A	15/04/92
		BR 9106839 A	20/07/93
		CA 2092615 A	14/03/92
		DE 69129988 D,T	18/03/99
		DK 219490 D	00/00/00
		EP 0548228 A,B	30/06/93
		SE 0548228 T3	
		ES 2121786 T	16/12/98
		FI 931124 A	12/05/93
		JP 6501153 T	10/02/94
		US 5869438 A	09/02/99
		US 5892013 A	06/04/99
		DK 219590 D	00/00/00
		DK 219690 D	00/00/00

ATENT COOPERATION TRI TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 08 February 2001 (08.02.01)	
International application No. PCT/DK00/00300	**Applicant's or agent's file reference** 5885
International filing date (day/month/year) 02 June 2000 (02.06.00)	**Priority date** (day/month/year) 02 June 1999 (02.06.99)
Applicant CALLISEN, Thomas, Hønger et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 November 2000 (27.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Copy for the designated Office (DO/US) PCT/DK00/00300

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

NOVOZYMES A/S
Patents
Krogshoejvej 36
DK-2880 Bagsværd
DANEMARK

Date of mailing (day/month/year)
09 January 2001 (09.01.01)

Applicant's or agent's file reference
5885

IMPORTANT NOTIFICATION

International application No.
PCT/DK00/00300

International filing date (day/month/year)
02 June 2000 (02.06.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
NOVO NORDISK A/S
Novo Allé
DK-2880 Bagsværd
Denmark

State of Nationality: DK
State of Residence: DK
Telephone No.: + 45 4444 8888
Facsimile No.: + 45 4442 6080
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
NOVOZYMES A/S
Krogshoejvej 36
DK-2880 Bagsværd
Denmark

State of Nationality: DK
State of Residence: DK
Telephone No.: + 45 4444 8888
Facsimile No.: + 45 4442 6080
Teleprinter No.:

3. Further observations, if necessary:
Please note that the Common Representative has also been changed accordingly.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
A. Karkachi
Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US)

PCT/DK00/00300

,TENT COOPERATION TRE. Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

NOVOZYMES A/S
Patents
Krogshoejvej 36
DK-2880 Bagsværd
DANEMARK

Date of mailing (day/month/year)
30 April 2001 (30.04.01)

Applicant's or agent's file reference
5885

IMPORTANT NOTIFICATION

International application No.
PCT/DK00/00300

International filing date (day/month/year)
02 June 2000 (02.06.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
NOVO NORDISK A/S
Novo Allé
DK-2880 Bagsværd
Denmark

State of Nationality: DK
State of Residence: DK
Telephone No.: + 45 4444 8888
Facsimile No.: + 45 4442 6080
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
NOVOZYMES A/S
Krogshoejvej 36
DK-2880 Bagsværd
Denmark

CORRECTED VERSION

State of Nationality: DK
State of Residence: DK
Telephone No.: + 45 4444 8888
Facsimile No.: + 45 4442 6080
Teleprinter No.:

3. Further observations, if necessary:
The correspondence address has also been changed accordingly.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
F. Baechler

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

003992996

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
5885-WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK00/00300	02/06/2000	02/06/1999
International Patent Classification (IPC) or national classification and IPC C12N9/20		
Applicant NOVOZYMES A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/11/2000	23.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Vollbach, S Telephone No. +49 89 2399 8715

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK00/00300

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-13 as originally filed

Claims, No.:

1-17 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-17
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-17
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item IV

Lack of unity of invention
see explanation under item V.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The modification of lipolytic enzymes by linking one or more hydrophobic groups to said enzyme is disclosed in several of the prior art documents cited in the search report (all documents categorized x cited in the search report e.g Proceedings of the 20th European Peptide Symposium, 4-9 September 1998, meeting date 1988, 667-9, VAN BINSBERGEN JAN et al., Editors GUENTHER JUNG, ERNST BAYER, 'Peptides 1988', pages 667-668. XP002931955. D2:'Transesterification of oil by fatty acid-modified lipase','MOTOTAKE MURAKAMI ET AL.','JAOCS','',70/6/00-00-1993,571-574).
Therefore none of the generally drafted claims fulfils the requirements of Articles 33.2 PCT.

Should the applicant intend to file an amended set of claims which shall be suitable to overcome the objections above, the following additional considerations should be taken into account:

Especially in view of the prior art, a meaningful identification of the contribution over said art must be accompanied by a clear characterisation of the parent enzyme and the kind of modification. In the absence of precisely characterising said enzyme especially definitions as can be found in e.g. claim 2 are totally meaningless (Article 6 PCT).
In addition, the maintenance of various alternatives (either in one claim or in several independent claims) will most probably give rise to objections for lack of unity (again in view of the prior art) (Rules 13.1-13.3 PCT).
Moreover, any "modified lipolytic enzyme" to be maintained in the claims should be accompanied by experimental results in order to substantiate a possible inventive activity.

Re Item VIII

Certain observations on the international application
see explanation under item V.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/75295 A1

(51) International Patent Classification[7]: C12N 9/20, C11D 3/386

(21) International Application Number: PCT/DK00/00300

(22) International Filing Date: 2 June 2000 (02.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 1999 00778 2 June 1999 (02.06.1999) DK
60/138,081 8 June 1999 (08.06.1999) US

(71) Applicant *(for all designated States except US)*: **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CALLISEN, Thomas, Hønger** [DK/DK]; Forchhammersvej 13, DK-1920 Frederiksberg C (DK). **PATKAR, Shamkant, Anant** [DK/DK]; Christoffers Allé 91, DK-2800 Lyngby (DK). **SVENDSEN, Allan** [DK/DK]; Bakkeledet 28, DK-3460 Birkerød (DK). **VIND, Jesper** [DK/DK]; Bagsværdvej 115, DK-2800 Lyngby (DK).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/75295 A1

(54) Title: CHEMICALLY MODIFIED LIPOLYTIC ENZYME

(57) Abstract: Lipolytic enzymes are chemically modified by covalently linking one or more (particularly 1-3) hydrophobic groups to the enzyme molecule. The chemical modification improves the performance of the lipolytic enzyme, e.g., in baking or in detergents.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00300

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 9/20, C11D 3/386
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the 20th European Peptide Symposium September 4-9, 1998, Meeting Date 1988, 667-9, Van Binsbergen, Jan et al, Editors Günther Jung, Ernst Bayer, "Peptides 1988", see pages 667- 668 and table 1	1-17
	--	
X	JAOCS, Volume 70, No 6, 1993, Mototake Murakami et al, "Transesterification of Oil by Fatty Acid-Modified Lipase", page 571 - page 574, see abstract	1,3-8,10-17
Y		2,9
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2000	09 -10- 2000
Name and mailing address of the ISA: Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Yvonne Siösteen/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00300

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOSCI. BIOTECH. BIOCHEM., Volume 59, No 5, 1995, Yoshinori Takahashi et al, "Characteristics of Lipase Modified with Water-soluble Acylating Reagents and Its Esterification Ability", page 809 - page 812, see abstract, page 809, left column, lines 23-34 and page 811, left column, lines 7-9	1,3-8,10-17
Y		2,9
	--	
X	PROG. BIOTECHNOL, Volume 15, 1988, F. J. Plou et al, "Stabilization of hydrolases by chemical modification with fatty acids or polyethylene glycol", page 115 - page 120, see page 118, first paragraph and table 1	1,3-8,10-17
Y		2,9
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Y	WO 9414963 A1 (UNILEVER PLC), 7 July 1994 (07.07.94), see page 5, lines 1-12 and claim 2	2,9
A		1,3-8,10-17
	--	
Y	WO 9535381 A1 (UNILEVER PLC), 28 December 1995 (28.12.95), see page 5, lines 16-21 and claim 2	2,9
A		1,3-8,10-17
	--	
A	WO 9205249 A1 (NOVO NORDISK A/S), 2 April 1992 (02.04.92), see abstract, claim 1, page 2, lines 7-20	1-17
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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International application No.
PCT/DK 00/00300

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9414963 A1	07/07/94	AU 5699994 A	19/07/94
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		HU 9501771 D	00/00/00
		JP 8504588 T	21/05/96
		PL 309388 A	02/10/95
		SK 79595 A	08/11/95
		ZA 9309415 A	15/06/95
WO 9535381 A1	28/12/95	AU 2884595 A	15/01/96
WO 9205249 A1	02/04/92	AT 169671 T	15/08/98
		AU 657278 B	09/03/95
		AU 8617291 A	15/04/92
		BR 9106839 A	20/07/93
		CA 2092615 A	14/03/92
		DE 69129988 D,T	18/03/99
		DK 219490 D	00/00/00
		EP 0548228 A,B	30/06/93
		SE 0548228 T3	
		ES 2121786 T	16/12/98
		FI 931124 A	12/05/93
		JP 6501153 T	10/02/94
		US 5869438 A	09/02/99
		US 5892013 A	06/04/99
		DK 219590 D	00/00/00
		DK 219690 D	00/00/00

CHEMICALLY MODIFIED LIPOLYTIC ENZYME

FIELD OF THE INVENTION

The present invention relates to a chemically modified lipolytic enzyme, its preparation and its to uses thereof.

BACKGROUND OF THE INVENTION

Lipolytic enzymes such as lipases and phospholipases are used, e.g., in detergents and baking.

Thus, lipases have been used for a number of years as detergent enzymes to remove lipid or fatty stains from clothes and other textiles, particularly a lipase derived from *Humicola lanuginosa* (EP 258 068 and EP 305 216) sold under the trade name Lipolase ® (product of Novo Nordisk A/S).

Fatty acid-modified lipases and their use in transesterification have been described. M. Murakami et al., JAOCS, 70 (6), 571-574 (1993); K. Green et al., JAOCS, 75 (11), 1519-1526 (1998).

It is also known to add lipases and phospholipases to breadmaking dough. WO 94/04035; WO 98/26057.

SUMMARY OF THE INVENTION

The inventors have developed lipolytic enzymes which are chemically modified by covalently linking one or more hydrophobic groups to the enzyme molecule. They found that the chemical modification may improve the performance of the lipolytic enzyme, e.g., in baking or in detergents. The benefits may include improved thermostability and an altered substrate specificity. A modified lipase or cutinase may show improved detergency, particularly improved first-wash performance, whiteness maintenance, dingy cleanup, and reduced formation of fatty acids during the drying process with less risk of forming an unpleasant smell. The benefits in baking include an increased loaf volume.

Accordingly, the invention provides a lipolytic enzyme which is chemically modified by having one or more (particularly 1-3) hydrophobic groups covalently

linked to the enzyme. The invention also provides us of such modified lipolytic enzyme in detergents and baking.

The invention further provides a method of preparing a chemically modified lipolytic enzyme by covalently linking hydrophobic groups to a parent lipolytic enzyme. Optionally, the amino acid sequence of the enzyme may be modified before the covalent linking.

DETAILED DESCRIPTION OF THE INVENTION

Parent lipolytic Enzyme

The lipolytic enzyme is an enzyme classified under the Enzyme Classification number E.C. 3.1.1.- (Carboxylic Ester Hydrolases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB). Thus, the lipolytic enzyme may exhibit hydrolytic activity, typically at a water/lipid interface, towards carboxylic ester bonds in substrates such as mono-, di- and triglycerides, phospholipids, thioesters, cholesterol esters, wax-esters, cutin, suberin, synthetic esters or other lipids mentioned in the context of E.C. 3.1.1. The lipolytic enzyme may, e.g., have activity lipase activity (with triglycerides as substrate), phospholipase activity, esterase activity or cutinase activity.

The parent lipolytic enzyme may be prokaryotic, particularly a bacterial enzyme, e.g. from *Pseudomonas*. Examples are *Pseudomonas* lipases, e.g. from *P. cepacia*, *P. glumae*, *P. pseudoalcaligenes* and *Pseudomonas sp.* strain SD 705. Other examples are bacterial cutinases, e.g. from *Pseudomonas* such as *P. mendocina* (US 5,389,536) or *P. putida* (WO 88/09367).

Alternatively, the parent lipolytic enzyme may be eukaryotic, e.g. fungal, such as lipolytic enzymes of the *Humicola* family and the *Zygomycetes* family and fungal cutinases. Examples of fungal cutinases are the cutinases of *Fusarium solani pisi* and *Humicola insolens*.

The *Humicola* family of lipolytic enzymes consists of the lipase from *H. lanuginosa* strain DSM 4109 and lipases having more than 50 % homology with said lipase. The lipase from *H. lanuginosa* (synonym *Thermomyces lanuginosus*) is de-

scribed in EP 258 068 and EP 305 216, and has the amino acid sequence shown in positions 1-269 of SEQ ID NO: 2 of US 5,869,438.

The *Humicola* family also includes the following lipolytic enzymes: lipase from *Penicillium camembertii*, lipase/phospholipase from *Fusarium oxysporum*, lipase from *F. heterosporum*, lysophospholipase from *Aspergillus foetidus*, phospholipase A1 from *A. oryzae*, lipase from *A.* oryzae, lipase/ferulic acid esterase from *A. niger*, lipase/ferulic acid esterase from *A. tubingensis*, lipase from *A. tubingensis*, lysophospholipase from *A. niger* and lipase from *F. solani*.

The *Zygomycetes* family comprises lipases having at least 50 % homology with the lipase of *Rhizomucor miehei*. This family also includes the lipases from *Absidia reflexa*, *A. sporophora*, *A. corymbifera*, *A. blakesleeana*, *A. griseola* and *Rhizopus oryzae*.

The phospholipase may have A_1 or A_2 activity to remove fatty acid from the phospholipid and form a lyso-phospholipid, or it may be have phospholipase B or lysophospholipase activity. It may or may not have lipase activity, i.e. activity on triglycerides. The phospholipase may be of animal origin, e.g. from pancreas (e.g. bovine or porcine pancreas), snake venom or bee venom. Alternatively, the phospholipase may be of microbial origin, e.g. from filamentous fungi, yeast or bacteria, such as the genus *Aspergillus, Fusarium* or *Hyphozyma* (WO 98/18912), particularly the species *A. niger* or *F. oxysporum* (WO 98/26057).

Other examples of lipolytic enzymes are described in PCT/DK 99/00664 (Danish patent application PA 1998 01572).

The lipolytic enzyme may be native to such source, or it may be a variant thereof obtained by altering the amino acid sequence. Examples of such variants are those described in WO 92/05249, WO 94/25577, WO 95/22615, WO 97/04079 and WO 97/07202, WO 98/08939, PCT/DK 99/00068, EP 99610010.3 and Danish patent application PCT/DK 00/00156 (PA 1999 00441). A specific example is a variant of the *Humicola lanuginosa* lipase having the mutations E1SPPCGRRP +E99N +N101S +E239C +Q249R.

Hydrophobic group

Generally, a hydrophobic group can be identified from a negative free-energy-of-transfer from water to oil. More specifically, suitable hydrophobic groups can be identified in a partition coefficient experiment where the two media are an aqueous detergent solution and a surface containing the (lipid) substrate of choice. The general concept is described in standard text books such as C. Tanford (1980), The hydrophobic effect, Wiley, New York.

The hydrophobic group may be a fatty acyl group, particularly having 12-22 or 14-20 carbon atoms, straight-chain or branched, saturated, mono- or polyunsaturated, optionally substituted. Examples are myristoyl (tetradecanoyl), palmitoyl (hexadecanoyl), stearoyl (octadecanoyl) and arachidoyl (eicosanoyl).

Other examples of hydrophobic groups are those commonly found in surfactants, e.g. a hydrophobic polymer group such as poly-alkoxy or alkyl-polyalkoxy of the general formula R^1-(O-CHR^2-CH_2)$_n$ wherein R^1 is H or C_{14}-C_{22} alkyl, R^2 is H or methyl, and n is 10-200, e.g. 20-100.

The hydrophobic group(s) may particularly be linked to an amino acid in the lipid contact zone of the lipolytic enzyme (as described in WO 92/05249) or within 5 Å from the edge of said zone.

The modified lipolytic enzyme containing one, two or three hydrophobic groups will be referred to as a monopod, dipod or tripod, respectively.

Covalent linking

The hydrophobic group may be covalently linked, e.g., to an amino group (lysine or N-terminal), a thiol group (cysteine residues), a hydroxyl group (serine or threonine) or a carboxyl group (glutamic acid, aspartic acid or C-terminal) in the amino acid sequence of the lipolytic enzyme. The covalent linking can be done by methods known in the art.

Thus, linking to amino groups can be done through a reactive intermediate such as an N-hydroxy-succinimide activated fatty acids, e.g. stearoyl or arachidoyl acid N-hydroxy-succinimide, or maleimide esters at high pH (e.g. pH 8-9).

Linking to a thiol group can be done by linking to a maleimide ester at pH 6.5-7, by reaction with fatty acid methane thiosulfonate (e.g. at pH 8), or as described in WO 91/16423, WO 98/23732 or WO 99/37323.

Linking to a carboxyl group can be done by linking a hydrophobic amine as described in WO 95/09909.

To ensure that the number of hydrophobic groups linked to each enzyme molecule will be from one to three, one strategy uses a lipolytic enzyme having an amino acid sequence with one, two or three of the group in question (e.g. amino or thiol). This is discussed below.

Another strategy is to choose the conditions (amounts of reagents etc.) for the linking reaction such that, on average, 1-3 hydrophobic groups will be linked to each enzyme molecule.

Amino acid sequence

A lipolytic enzyme with 1-3 groups may be a variant obtained by modifying the amino acid sequence of a given lipolytic enzyme by recombinant technology using site-directed mutagenesis.

Thiol groups can also be introduced by chemical reaction as described in Duncan et al., (1983) Anal. Biochem.132, 68-73.

The N-terminal amino group may be eliminated by using site-directed mutagenesis to change the N-terminal to glutamine and after expression convert this to pyroglutamate by cyclization (Thiede,B, Lamer S., Mattow J., Siejak F., Dimmler C., Rudel T., Jungblut PR.; rapid communications in Mass spectroscopy Vol 14 (6) pp.496-502 (2000). A choice for expression of pyroglutamate containing peptide in filamentous fungi, could be to use parts of the signal peptide and N-terminal of the peroxidase from the filamentous fungi *Coprinus cinereus*. This peroxidase has an N-terminal pyroglutamate (Baunsgaard L., Dalboge H., Houen G., Rasmussen EM, Welinder KG:, European journal of Biochemistry vol. 213 (1) 605-611 (1993).

The peroxidase N-terminal and part of the neighboring amino acids can be conferred to the N-terminal of the lipolytic enzyme by standard molecular biological techniques to created a variant with a pyro-glutamic N-terminal.

Lipolytic enzyme variant

The lipolytic enzyme variant may be designed to change the number and location of amino or thiol groups by amino acid insertion, deletion and/or substitution involving lysine or cysteine.

A change in the number of lysine residues may be balanced by a change in the number of other charged amino acids may, to keep the isoelectric point fairly unchanged. Thus, lysine may be substituted with another positively charged amino acid (histidine or arginine).

One strategy is to remove some of the lysine residues by substitution or deletion and keep 1-3 lysine residues unchanged. Thus, of the 6 lysine residues in the *Humicola lanuginosa* lipase, one or more of the following may be retained: K24, K98, K233.

Another strategy is to remove all lysine residues in the native lipolytic enzyme by substitution or deletion (and optionally remove the N-terminal amino group) and to introduce one, two or three lysine residues by substitution or insertion at selected positions in the lipid contact zone.

Thus, for a lipolytic enzyme of the *Humicola* family, existing amino groups may be removed, and 1-3 lysine residues may be introduced at positions corresponding to the following amino acids in the *Humicola lanuginosa* lipase: 14, 15, 17-28, 35-42, 45, 54-65, 80-85, 87-95, 110-116, 119, 144-151, 171-177, 195-209, 213-215, 219, 221-231, 234, 238, 242-251, 257-269, particularly at position 199, 56, 27, 111, 118, 37, 227, 226, 210, 95, 93, 255, 96, 252, 57 or 211.

Similarly, for a fungal cutinase, existing amino groups may be removed and 1-3 lysine residues may be introduced, e.g. by substitutions corresponding to I5K, V158K, D63K, N44K and/or R149K. Examples are I5K +V158K +D63K and N44K +V158K +D63K.

Use of modified lipolytic enzyme

The modified lipolytic enzyme can be used in any known application for such enzymes, e.g. in baking, in detergents or in immobilized form for various processes.

Baking

The modified lipolytic enzyme can be used in the preparation of dough, bread and cakes, e.g. to increase dough stability and dough handling properties, to increase the loaf volume or to improve the elasticity of the bread or cake. Thus, the enzyme can be used in a process for making bread, comprising adding the enzyme to the ingredients of a dough, kneading the dough and baking the dough to make the bread. This can be done in analogy with US 4,567,046 (Kyowa Hakko), JP-A 60-78529 (QP Corp.), JP-A 62-111629 (QP Corp.), JP-A 63-258528 (QP Corp.), EP 426211 (Unilever) or WO 99/53769 (Novo Nordisk).

Detergent

The lipolytic enzyme (e.g. a lipase) may be used as an additive in a detergent composition. This additive is conveniently formulated as a non-dusting granulate, a stabilized liquid, a slurry or a protected enzyme. The additive may be prepared by methods known in the art.

Lipases tend to exert the best fat removing effect after more than one wash cycle (Gormsen et al., in Proceedings of the 3rd World Conference on Detergents, AOCS press, 1993, pp 198-203).

Immobilized enzyme

The lipolytic enzyme may be immobilized by methods known in the art, e.g. by adsorption onto a polymer based carrier, by covalent binding to an activated polymer-based carrier (e.g. epoxy or aldehyde) and by granulation, e.g. as described in WO 89/02916, WO 90/15868, WO 95/22606 or WO 99/33964.

The immobilized lipolytic enzyme may be used for interesterification, e.g. of a water-insoluble carboxylic acid ester (such as a triglyceride) with another ester, with a free fatty acid or with an alcohol. The immobilized enzyme can also be used in ester synthesis or in resolution of racemic compounds.

DETERGENT COMPOSITION

The detergent compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the pretreatment of stained fab-

rics, rinse added fabric softener compositions, and compositions for use in general household hard surface cleaning operations and dishwashing operations.

The detergent composition of the invention comprises the lipase of the invention and a surfactant. Additionally, it may optionally comprise a builder, another enzyme, a suds suppresser, a softening agent, a dye-transfer inhibiting agent and other components conventionally used in detergents such as soil-suspending agents, soil-releasing agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or non-encapsulated perfumes.

The detergent composition according to the invention can be in liquid, paste, gels, bars or granular forms. The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. in the range of 7-11, particularly 9-11. Granular compositions according to the present invention can also be in "compact form", i.e. they may have a relatively higher density than conventional granular detergents, i.e. form 550 to 950 g/l.

The lipase of the invention, or optionally another enzyme incorporated in the detergent composition, is normally incorporated in the detergent composition at a level from 0.00001% to 2% of enzyme protein by weight of the composition, particularly at a level from 0.0001% to 1% of enzyme protein by weight of the composition, more particularly at a level from 0.001% to 0.5% of enzyme protein by weight of the composition, even more particularly at a level from 0.01% to 0.2% of enzyme protein by weight of the composition.

The detergent composition of the invention may comprise the lipase in an amount corresponding to 10-50,000 LU per gram of detergent, particularly 20-5,000 LU/g, e.g. 100-1000 LU/g. The detergent may be dissolved in water to produce a wash liquor containing lipolytic enzyme in an amount corresponding to 25-15,000 LU per liter of wash liquor, particularly 100 - 5000 LU/l, e.g. 300-2000 LU/l. The amount of lipase protein may be 0.001-10 mg per gram of detergent or 0.001-100 mg per liter of wash liquor.

More specifically, the lipase of the invention may be incorporated in the detergent compositions described in WO 97/04079, WO 97/07202, WO 97/41212, PCT/DK WO 98/08939 and WO 97/43375.

Surfactant system

The surfactant system may comprise nonionic, anionic, cationic, ampholytic, and/or zwitterionic surfactants. The surfactant system may comprise a combination of anionic and nonionic surfactant with 70-100 % by weight of anionic surfactant and 0-30 % by weight of nonionic, particularly 80-100 % of anionic surfactant and 0-20 % nonionic or 40-70 % anionic and 30-60 % non-ionic surfactant.

The surfactant is typically present at a level from 0.1% to 60% by weight, e.g. 1% to 40%, particularly 10-40 %. particularly from about 3% to about 20% by weight. Some examples of surfactants are described below.

Anionic surfactants

Suitable anionic surfactants include alkyl sulfate, alkyl ethoxy sulfate, linear alkyl benzene sulfonate and mixtures of these.

The alkyl sulfate surfactants are water soluble salts or acids of the formula $ROSO_3M$ wherein R particularly is a C_{10}-C_{24} hydrocarbyl, particularly an alkyl or hydroxyalkyl having a C_{10}-C_{20} alkyl component, more particularly a C_{12}-C_{18} alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium.

Alkylbenzene sulfonates are suitable, especially linear (straight-chain) alkyl benzene sulfonates (LAS) wherein the alkyl group particularly contains from 10 to 18 carbon atoms.

Suitable anionic surfactants include alkyl alkoxylated sulfates which are water soluble salts or acids of the formula $RO(A)_mSO_3M$ wherein R is an unsubstituted C_{10}-C_{-24} alkyl or hydroxyalkyl group having a C_{10}-C_{24} alkyl component, particularly a C_{12}-C_{20} alkyl or hydroxyalkyl, more particularly C_{12}-C_{18} alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more particularly between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and di-

methyl piperdinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like.

Other anionic surfactants include salts (including, for example, sodium, potassium, ammonium, and substituted ammonium salts such as mono- di- and triethanolamine salts) of soap, C_8-C_{22} primary or secondary alkanesulfonates, C_8-C_{24} olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates.

Nonionic surfactant

The surfactant may comprise polyalkylene oxide (e.g. polyethylene oxide) condensates of alkyl phenols. The alkyl group may contain from about 6 to about 14 carbon atoms, in a straight chain or branched-chain. The ethylene oxide may be present in an amount equal to from about 2 to about 25 moles per mole of alkyl phenol.

The surfactant may also comprise condensation products of primary and secondary aliphatic alcohols with about 1 to about 25 moles of ethylene oxide. The alkyl chain of the aliphatic alcohol can either be straight or branched, and generally contains from about 8 to about 22 carbon atoms.

Further, the nonionic surfactant may comprise polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide, alkylpolysaccharides, and mixtures hereof, particularly C_8-C_{14} alkyl phenol ethoxylates having from 3 to 15 ethoxy groups and C_8-C_{18} alcohol ethoxylates (particularly C_{10} avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Examples of nonionic surfactants are alcohol ethoxylate, alcohol phenol ethoxylate, polyhydroxy fatty acid amide, alkyl polyglucoside and mixtures of these.

EXAMPLES

Example 1: Modified lipases with an average of 3 hydrophobic groups

Modified lipases were prepared by covalently linking tetradecanoyl (C_{14}) and hexadecanoyl (C_{16}) groups, respectively, to Lipolase (*Humicola lanuginosa* lipase). Each lipase molecule has 7 amino groups (N-terminal + 6 lysine resi-

dues), and it was estimated that an average of 3 fatty acyl groups were linked to each molecule.

Example 2: Modified lipases with 3 or 4 hydrophobic groups

Two variants of Lipolase were prepared by amino acid substitutions so that the variants had the following amino groups. Other lysine residues were substituted with arginine:

Three amino groups N-terminal and lysine at positions 46 and 98.

Four amino groups: N-terminal and lysine at positions 24, 46 and 98.

Fatty acyl groups (myristoyl and stearoyl, respectively) were linked covalently to the amino groups in each variant.

Example 3: Modified lipases with 2 hydrophobic groups

A variant of Lipolase was prepared by substituting lysine residues with arginine to obtain a lipase variant having two amino groups, at the N-terminal and Lys 24.

Four different modified lipases were produced by linking the following hydrophobic groups to the amino groups in the variant:

Stearoyl

$C_{18}H_{37}$-(O-CH_2-CH_2)$_{100}$

$C_{18}H_{37}$-(O-CH_2-CH_2)$_{21}$

Arachidoyl

A similar modified lipase may be made by linking to palmitoyl groups.

Example 4: Construction of modified lipases

Monopods, dipods and tripods are prepared from Lipolase by removing the N-terminal amino group by pyroglutamate cyclization and making variants by amino acid substitutions having lysine at the following positions. Other lysine residues are substituted with arginine:

Monopod: lysine at position 98, 211 or 223.

Dipod: lysine residues at positions 98 +233 or 96 +255.

Tripod: Lysine residues at positions 24 +98 +223 or at positions 57 +96 +252.

Hydrophobic groups (fatty acyl or polypropylene) are linked covalently to the lysine residues in each variant.

Example 5: First-Wash Performance

The two modified lipases were tested as described below, and unmodified Lipolase was tested for comparison.

A number of variants according to the invention were tested in an anionic detergent. The experimental conditions were as follows:

Equipment:	Thermostated Terg-o-tometer
Method:	1 cycle wash followed by line drying.
Wash liquor:	1000 ml per beaker
Swatches:	7 (cotton style # 400) swatches (9*9 cm) per beaker.
Stain:	Lard coloured with Sudan red (0,75mg Sudan red/g lard). 250 µl of lard/Sudan red heated to 70°C is applied to the center of each swatch, followed by line-drying over-night.
Water:	8.4° German hardness (°dH), Ca : Mg = 2:1
Detergent:	1.8 g/l commercial detergent (Wisk)
Lipase dosage:	as indicated below
Wash time:	20 min.
Temperature:	30°C
Rinse:	15 minutes in running tap water.
Drying:	Overnight at room temperature (~ 20°C, 30-40 % RH).
Evaluation:	The reflectance was measured at 460 nm in a reflectometer. The results are given as ΔR (delta Reflectance) = reflectance of swatches washed in detergent with lipase minus reflectance of swatches washed in detergent without lipase.

Results:

	Lipase	Dosage, LU/l	ΔR
Reference	Lipolase	1329	0.3
		4011	0.7
Invention	Lipolase modified with C_{14}	1617	1.9
		4880	5.2
	Lipolase modified with C_{16}	1212	2.2
		3658	5.5

The results clearly demonstrate that the modified lipases have an improved first-wash performance.

Example 6: Baking tests

A chemically modified lipase was prepared by linking palmitoyl groups to Humicola lanuginosa lipase. The amounts of reagents were chosen so as to link an average of 2-3 acyl groups to each lipase molecule.

The chemically modified lipase was compared the unmodified lipase in a traditional European straight dough baking procedure.

The volume and the shape of the rolls were evaluated. Volume was evaluated by simple displacement of 10 rolls, and the shape was evaluated by measuring height/width. The results were as follows

	Invention (modified lipase)	Reference (unmodified lipase)
Lipase dosage, LU/kg flour	500	1000
Volume, ml/g	6.4	6.2
Shape (Height/width)	0.68	0.64

The results clearly show that the modified lipase at half the dosage of the reference has improved performance in terms of volume and shape.

CLAIMS

1. A lipolytic enzyme which is chemically modified by having one, two or three hydrophobic groups covalently linked to a parent lipolytic enzyme.

2. The lipolytic enzyme of claim 1 which has a hydrophobic group covalently linked to an amino acid located in the lipid contact zone of the parent lipolytic enzyme or within 5 Å from the edge of said zone.

3. The lipolytic enzyme of claim 1 or 2 wherein the parent lipolytic enzyme has an amino acid sequence having one, two or three amino groups, and wherein the hydrophobic group(s) is/are covalently linked to the amino group(s).

4. The lipolytic enzyme of any preceding claim wherein the hydrophobic group is a fatty acyl group, a polyalkoxy or an alkyl-polyalkoxy group.

5. The lipolytic enzyme of any preceding claim wherein the parent lipolytic enzyme belongs to the *Humicola* group, particularly *Humicola lanuginosa* lipase.

6. The lipolytic enzyme of any preceding claim wherein the lipolytic enzyme is a lipase, a cutinase or a phospholipase.

7. A method of preparing a chemically modified lipolytic enzyme, comprising covalently linking hydrophobic groups to a parent lipolytic enzyme so as to link an average of 1-3 hydrophobic groups to each enzyme molecule.

8. A method of preparing a chemically modified lipolytic enzyme, comprising:

a) modifying a parent lipolytic enzyme so as to change the number and/or positions of amino, thiol, hydroxy or carboxy groups, and

b) covalently linking hydrophobic groups to the amino, thiol, hydroxy or carboxy groups.

9. The method of the preceding claim wherein the modification results in one, two or three amino, thiol, hydroxy or carboxy groups, particularly located in the lipid contact zone of the parent lipolytic enzyme.

10. The method of claim 8 or 9 wherein the modification comprises modification of the amino acid sequence by site-directed mutagenesis.

11. The method of any of claims 8-10 wherein the modification comprises substitution of a lysine residue with another amino acid (particularly arginine or histidine) and/or substitution of another amino acid residue with lysine and/or chemical modification to remove the N-terminal amino group, and the hydrophobic group is linked to amino groups.

12. The method of the preceding claim wherein the modification results in 1-3 amino groups in the enzyme molecule.

13. A detergent composition comprising a surfactant and a lipolytic enzyme which has at least one hydrophobic group covalently linked.

14. The detergent composition of the preceding claim wherein the hydrophobic group is a fatty acyl group.

15. The detergent composition of claim 13 or 14 wherein the lipolytic enzyme has one, two or three hydrophobic groups covalently linked.

16. A method of preparing a dough or a baked product from the dough which comprises adding to the dough a lipolytic enzyme which has at least one hydrophobic group covalently linked.

17. A dough composition comprising a lipolytic enzyme which has at least one hydrophobic group covalently linked.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00300

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 9/20, C11D 3/386
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the 20th European Peptide Symposium September 4-9, 1998, Meeting Date 1988, 667-9, Van Binsbergen, Jan et al, Editors Günther Jung, Ernst Bayer, "Peptides 1988", see pages 667- 668 and table 1	1-17
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X	JAOCS, Volume 70, No 6, 1993, Mototake Murakami et al, "Transesterification of Oil by Fatty Acid-Modified Lipase", page 571 - page 574, see abstract	1,3-8,10-17
Y		2,9
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2000	09 -10- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Yvonne Siösteen/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00300

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOSCI. BIOTECH. BIOCHEM., Volume 59, No 5, 1995, Yoshinori Takahashi et al, "Characteristics of Lipase Modified with Water-soluble Acylating Reagents and Its Esterification Ability", page 809 - page 812, see abstract, page 809, left column, lines 23-34 and page 811, left column, lines 7-9	1,3-8,10-17
Y		2,9
	--	
X	PROG. BIOTECHNOL, Volume 15, 1988, F. J. Plou et al, "Stabilization of hydrolases by chemical modification with fatty acids or polyethylene glycol", page 115 - page 120, see page 118, first paragraph and table 1	1,3-8,10-17
Y		2,9
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Y	WO 9414963 A1 (UNILEVER PLC), 7 July 1994 (07.07.94), see page 5, lines 1-12 and claim 2	2,9
A		1,3-8,10-17
	--	
Y	WO 9535381 A1 (UNILEVER PLC), 28 December 1995 (28.12.95), see page 5, lines 16-21 and claim 2	2,9
A		1,3-8,10-17
	--	
A	WO 9205249 A1 (NOVO NORDISK A/S), 2 April 1992 (02.04.92), see abstract, claim 1, page 2, lines 7-20	1-17
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